US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for AOAC Use Dilution Method for Testing Disinfectants

SOP Number: MB-05-05

Date Revised: 10-16-07

Superseded SOP: MB 05-04 AOAC Use Dilution Method for Testing Disinfectants

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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Initiated By: _____ Date: ___/___/___

Print Name:_____

Technical Review: _____ Date: ___/___/___

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Date Issued: ___/___/___

Controlled Copy No.: _____

Withdrawn By: _____ Date: ___/___/___

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION:

1.1 This SOP describes the methodology used to determine the efficacy of disinfectants against two organisms, *Pseudomonas aeruginosa and Staphylococcus aureus* on hard surfaces. Several aspects of AOAC methods 955.15 (Testing Disinfectants against *Staphylococcus aureus*) and 964.02 (Testing Disinfectants against *Pseudomonas aeruginosa*) have been officially modified (editorial modifications) to improve the quality and reproducibility of the efficacy data generated from the method. The revised methods appear in: Official Methods of Analysis (2006) 18th ED., AOAC INTERNATIONAL, Methods 955.15 and 964.02, Gaithersburg, MD, Chapter 6.

2.0 DEFINITIONS:

2.1 AOAC = AOAC INTERNATIONAL

2.2 OD = outside diameter

2.3 ID = inside diameter

2.4 References to water mean reagent-grade water, except where otherwise specified.

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organisms are required to be performed in accordance to biosafety practices stipulated in SOP MB-01, Lab Biosafety.

3.2 Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, phenol, etc. Personal protective clothing or devices are recommended during the handling of these items for the purpose of activation, dilution, or efficacy testing. A chemical fume hood or other containment equipment is employed when performing tasks with concentrated products. The study analyst may wish to consult the Material Safety Data Sheet for the specific product/active ingredient to determine the best course of action.

4.0 CAUTIONS:

4.1 Follow appropriate chain-of-custody (COC) guidelines during testing as stipulated in SOP COC-01, Sample Log-in and Tracking.

4.2 Strict adherence to the protocol is necessary for the validity of the test results.

4.3 To ensure the stability of a diluted product, prepare the dilutions within three hours of the disinfectant treatment step unless specified otherwise.

4.4 Use appropriate aseptic techniques for all test procedures involving the manipulation of the test organisms and associated test components.

4.5 These microbiological methods are very technique-sensitive and technique-oriented; thus, exact adherence to the method, good laboratory practices, and quality control are required for proficiency and validity of the results.

4.6 Detergents used in washing glassware may leave residues which are bacteriostatic. Test for inhibitory residues on glassware periodically according to SOP QC-03, Glass Washing and Detergent Residues Test.

4.7 The primary subculture medium should serve as a suitable neutralizer for the test substance as well as an adequate growth medium which must be confirmed in advance or concurrently with the use dilution test.

4.7.1 See SOP MB-17, Neutralization Confirmation, for the procedure to determine suitability of the neutralizer (primary subculture tube) for the test substance.

4.7.2 See SOP QC-11, Performance Assessment and Sterility Verification, to determine whether or not the subculture medium is an adequate growth medium.

5.0 INTERFERENCES:

5.1 Touching the interior sides of the medication tube should be avoided while the carriers are being lowered into the disinfectant agent and the hook is being removed. Contact with the interior sides of the medication tube may cause adhesion of bacterial cells which are not in contact with the disinfectant. This may result in re-inoculation of the carriers with organism as they are being removed from the medication tube. Re-inoculation of the carriers with organism can lead to false positive results.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

6.2 The laboratory staff shall confirm (i.e., documentation in the training file of familiarization with the SOP) that they can properly perform the procedure before

commencing work. If the standard AOAC method changes, confirmation shall be repeated.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 *Test organisms. Staphylococcus aureus* (ATCC No. 6538) and *Pseudomonas aeruginosa* (ATCC No. 15442) obtained directly from a reputable supplier (e.g., ATCC).

7.2 *Culture media* (e.g., nutrient broth, synthetic broth, nutrient agar). Note: Commercial dehydrated media made to conform to the recipes provided in AOAC Methods 955.15 and 964.02 may be substituted.

7.3 *Subculture media* (e.g., letheen broth, fluid thioglycollate medium). Note: Commercial dehydrated media made to conform to the recipes provided in AOAC Methods 955.15 and 964.02 may be substituted.

7.4 *Sterile water.* Use reagent-grade water. Reagent-grade water should be free of substances that interfere with analytical methods. Any method of preparation of reagent-grade water is acceptable provided that the requisite quality can be met. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent-grade water when used in the proper arrangement. See Standard Methods for the Examination of Water and Wastewater and SOP QC-01, Quality Assurance of Purified Water for details on reagent-grade water.

7.5 *Carriers*. Polished stainless steel cylinders, 8 ± 1 mm OD, 6 ± 1 mm ID, 10 ± 1 mm length; type 304 stainless steel, SS 18-8 (S & L Aerospace Metals, Maspeth, NY or Fisher Scientific catalog number 7-907-5 as of January 2006).

7.6 *Glassware.* For disinfectant, use autoclavable 25 × 150 mm or 25 × 100 mm tubes (Bellco Glass Inc., Vineland, NJ). For cultures/subcultures, use autoclavable reusable or disposable 20 x 150 mm tubes. For stock cultures, use 16 x 100 mm screw cap tubes. Cap tubes with closures before sterilizing. Sterilize all glassware in hot air oven at 180°C or steam sterilize for a minimum of 20 minutes at 121°C with drying cycle.

7.7 *Water bath/chiller unit.* Constant temperature for test chemical, capable of maintaining 20 ± 1°C temperature or specified temperature for conducting the test.

7.8 *Test tube racks.* Any convenient style.

7.9 *Transfer loops.* Make 4 mm ID single loop at end of 50–75 mm (2–3 in.) Pt or Pt alloy wire No. 23 B&S gage or 4 mm loop fused on 75 mm (3 in.) shaft (available from Johnson Matthey, West Chester, PA 19380, USA). Fit other end in suitable holder. Bend loop at 30° angle with stem. Volumetric transfer devices may be used instead of transfer loops (e.g., micro volume pipet).

7.10 *Wire Hook*. For carrier transfer. Make 3 mm right angle bend at end of 50–75 mm nichrome wire No. 18 B&S gage. Place other end in suitable holder.

7.11 *Timer.* For managing timed activities, any certified timer that can display time in seconds.

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Disinfectants are stored according to manufacturers' recommendations or at room temperature if the product label or testing parameters do not identify a storage temperature. Those disinfectants requiring activation or dilution prior to use will only be activated or diluted within three hours of testing unless test parameters specify otherwise.

10.0 PROCEDURE AND ANALYSIS:

10.1 Brief Summary: The AOAC Use-dilution test is a carrier-based test. Carriers (stainless steel cylinders) are inoculated with a test organism, dried, exposed to the use-dilution of the disinfectant product, and cultured to assess the survival of the bacteria. A single test involves the evaluation of 60 inoculated carriers (one organism) against one product sample. In addition to the 60 carriers, 6 carriers are required to estimate carrier bacterial load and a minimum of 6 more are included as extras. Thus, a minimum of 72 seeded carriers are required to perform a single test.

10.2 Test Culture Preparation:

10.2.1 Initiate test culture by inoculating a 10 mL tube (20 × 150 mm) of nutrient broth or synthetic broth from a stock slant or stab culture. Transfer one 4 mm ID loopful (or use a 10 µL certified

transfer loop) of inoculum from the stock culture into the broth. Refer to SOP MB-02, Test Microbes.

10.2.2 Two sets of cultures (one set as a backup) of the same organism may be initiated in parallel from the same stock culture and subcultured; however, only one set of the final cultures is used for actual testing. Select set with typical growth.

10.2.3 Make at least 3 consecutive 24 ± 2 hour transfers (use one 4 mm ID loopful, or a 10 µL certified transfer loop, or a calibrated micro volume pipet to deliver 10 µL) in 10 mL nutrient broth or synthetic broth incubated at 36 ± 1°C. Up to 30 ± 2 total transfers are allowed. If only one of the consecutive 24 hour transfers has been missed, it is not necessary to repeat the previous 3-day sequence prior to the inoculation of the 48–54 hour test culture.

10.2.4 For the final subculture step, inoculate for the test procedure, a sufficient number of 25 × 150 mm tubes (e.g., eight to ten) containing 20 mL nutrient or synthetic broth; incubate 48–54 hours at 36 ± 1°C.

10.2.5 A minimum of five days are required to obtain the culture for seeding carriers. For example, the culture sequence must begin on Thursday for testing to commence on the following Tuesday.

10.2.6 Record all culture transfers on the Organism Culture Tracking form (see SOP MB-02, Test Microbes).

10.3 Carrier Inoculation for *S. aureus* and *P. aeruginosa*:

10.3.1 For *S. aureus*, using a Vortex-style mixer, mix 48-54 hour nutrient broth test cultures 3–4 seconds and let stand 10 minutes at room temperature before continuing. Remove the upper portion of each culture (e.g., upper ¾ or 15 mL), leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Aliquot 20 mL portions into sterile 25 × 150 mm test tubes. Prepare a minimum of four tubes.

10.3.2 For *P. aeruginosa*, do not shake 48–54 hour test culture. The pellicle from the 48–54 hour cultures must be removed from the broth before mixing on a Vortex mixer either by decanting the liquid aseptically into a sterile tube or by gently aspirating the

broth away from the pellicle using a pipet. Any disruption of the pellicle resulting in dropping, or breaking up of the pellicle in culture before or during its removal renders that culture unusable in the use-dilution test. Once the pellicle is removed, using a vortex-style mixer, mix nutrient broth test cultures 3–4 seconds and let stand 10 minutes at room temperature before continuing. Remove the upper portion of each culture (e.g., upper ¾ or 15 mL), leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix. Aliquot 20 mL portions into sterile 25 × 150 mm test tubes. Prepare a minimum of four tubes.

10.3.3 If organic burden is required for testing, the appropriate amount of organic burden is added to the test culture prior to the inoculation of carriers. For a 5% v/v preparation, add 1 mL organic burden per 19 mL test culture (e.g., add 5 mL organic burden to 95 mL test culture). Swirl to mix. Aliquot 20 mL portions into sterile 25 × 150 mm test tubes. Prepare a minimum of four tubes.

10.3.4 Use only carriers that have been physically screened, have passed biosreening, and have been appropriately prepared (see SOP MB-03, Screening Carriers).

10.3.5 Using a sterile hook, aseptically transfer 20 carriers prepared as described above into each of the tubes containing the test culture. Drain the water from the carriers by tapping them against the side of the tube before transferring. Multiple carriers may be transferred on a single wire hook. The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook. Be sure to inoculate a sufficient number of carriers for the test. (Alternately, the water may be siphoned off the carriers and the 20 mL test culture added directly to the carriers without transferring).

10.3.6 After 15 ± 2 min contact period, remove carriers using flamed nichrome wire hook; shake carrier vigorously against side of the tube to remove excess culture, and place on end in vertical position in sterile Petri dish matted with 2 layers of Whatman No. 2 (or equivalent) filter paper, making sure that carriers do not touch to prevent improper drying. Place no more than 12 carriers in a Petri dish. Carriers that touch or fall over cannot be used for testing and must be removed and recleaned. Once all of the carriers have been

transferred, cover and place in incubator at 36 ± 1°C and let dry 40 ± 2 min. Inoculated carriers must be used on day of preparation, preferably within two hours post-drying.

10.3.7 Record the timed carrier inoculation activities on the Time Recording Sheet for Carrier Inoculation Steps (see 16.1).

10.4 Disinfectant Sample Preparation:

10.4.1 Equilibrate the water bath and allow it to come to 20 ± 1°C or the temperature specified (±1°C). Prepare the disinfectant dilutions within 3 hours of performing the assay unless test parameters specify otherwise. Ready-to-use products are tested as received; no dilution is required.

10.4.2 Prior to opening the container of a liquid product, gently shake the container and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry. Remove the cap. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the lip of the spout with sterile instruments (i.e., razor blade, forceps).

10.4.3 Pour an appropriate aliquot of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place cap on the product container and secure tightly. From the beaker, dispense ready-to-use products directly into sterile medication tubes or initiate dilutions for concentrated products.

10.4.4 Aseptically prepare disinfectant samples as directed by the test parameters. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use ≥ 1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to 2 decimal places toward a stronger product.

10.4.5 Dispense 10 mL aliquots of the diluted disinfectant or ready-to-use product into 25 × 100 mm (or 25 × 150 mm) test tubes, one tube per carrier. Place tubes in the equilibrated water bath for approximately 10 minutes to allow test solution to come to specified temperature. Record the temperature of the water bath

and recirculating chiller before and after testing on the AOAC Use-Dilution Test Information Sheet (see 16.3).

10.4.6 Record disinfectant preparation on the Media/Reagent Preparation Sheet (see SOP QC-15, Media Prep and Sterilization Run Numbers). For one Use-dilution test, prepare approximately 1 L of the disinfectant.

10.5 Test Procedure:

10.5.1 After the required drying time, the carriers are sequentially transferred from the Petri dish to the test tubes containing the disinfectant at appropriate intervals. Use a certified timer to time the transfers. Modify intervals to accommodate exposure times other than 10 min.

10.5.2 One carrier is added per tube. Immediately after placing carrier in the test tube, briefly swirl tube before placing it back in the bath. For a contact time of ten minutes, the carrier must be deposited in the tube within ± 5 seconds of the prescribed drop time. For contact times of less than ten minutes, the analyst will work with the team leader and senior scientist to identify an appropriate (i.e., shorter) drop interval. Using alternating hooks, flame-sterilize the hook and allow it to cool after each carrier transfer.

10.5.3 When lowering the carriers into the disinfectant tubes, neither the carrier itself nor the tip of the wire hook can touch the interior sides of the tube. Individual manipulation of carriers is required; the use of semi-automated ring carrier is prohibited. (*Note*: Above step is one of the most critical, technique-sensitive areas of method. False positives can result from transfer of live organisms to sides of tubes due to contact or aerosol formation). If the side is touched, mark or note the tube; the tube is not counted if it yields a positive result.

10.5.4 After the carriers have been deposited into the disinfectant, and the exposure time is complete, the carriers are then transferred in a sequentially timed fashion into the primary subculture tubes containing the appropriate neutralizer (10 mL in 20 × 150 mm tubes). The carrier is removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant and transferred into the subculture tube.

10.5.5 Flame-sterilize the hook after each carrier transfer.

10.5.6 The remaining carriers are transferred into their corresponding primary subculture tubes at the appropriate time. As with the transfers to the disinfectant tubes, transfers into subculture tubes should be within ± 5 seconds (see section 10.5.2) of the actual transfer. Contact of the carrier to the interior sides of the subculture tube during transfer should be avoided as much as possible.

10.5.7 After the carrier is deposited in the subculture tube, recap the subculture tube and shake thoroughly. Place subculture tubes into 36 ± 1°C incubator.

10.5.8 After a minimum of 30 ± 5 minutes from the end of the transfer into primary subculture tubes, remove tubes from the incubator and transfer carrier from the primary tube to a secondary tube of sterile medium. Transfer the carriers using a sterile wire hook to a second subculture tube containing 10 mL of the appropriate subculture medium which may contain a suitable neutralizer. Move the carriers in order but the movements do not have to be timed. Thoroughly shake the subculture tubes after all of the carriers have been transferred.

10.5.9 Check all test tube racks for proper transfer of carriers (i.e., carriers are in the correct tubes, no tubes have two carriers) before completing the testing day.

10.5.10 Incubate both the primary and secondary subculture tubes 48 ± 2 hours at 36 ± 1°C.

10.5.11 Record timed events on the Time Recording Sheet for Carrier Transfer Form (see 16.2).

10.5.12 See Attachment A (Testing Footnotes and Explanations) for a list of footnotes which may be used to indicate problematic events or observations which occurred during testing.

10.6 Viability controls. On testing day, place a dried inoculated carrier into a tube containing 10 mL primary subculture medium and a second dried, inoculated carrier into a tube containing 10 mL secondary subculture medium. Incubate

tubes for 48 ± 2 hours at 36 ± 1°C. Positive growth in both tubes validates the test system. Failure to have growth in either of the tubes invalidates the test.

10.7 The bacterial carrier load on six carriers is assayed as stipulated in SOP MB-04, Carrier Counts.

10.8 Results:

10.8.1 Report results as + (growth), or 0 (no growth) as determined by presence or absence of turbidity, on the AOAC Use-dilution Results Sheet (see 16.4). A positive result is one in which the broth culture appears turbid. A negative result is one in which the broth appears clear. Each tube is shaken prior to recording results to determine the presence or absence of turbidity. The primary and secondary subculture tubes for each carrier represent a Acarrier set."

10.8.2 A positive result in either the primary or secondary subculture tube is considered a positive result for a carrier set.

10.8.3 In the event that there are positive carriers present in the test, the test may be repeated in order to confirm the outcome.

10.8.4 Once the results are recorded, it is important that the carriers be reprocessed before use in another study.

10.9 Confirmation Steps:

10.9.1 A minimum of three positive carrier sets per test, if available, should be confirmed using Gram staining, solid media, and VITEK™ analysis. If there are less than three positive carrier sets, then each carrier set will be confirmed. If both tubes are positive in a carrier set, only one tube is selected for confirmatory testing (preferably the secondary subculture tube with carrier).

10.9.2 For a test with greater than 20 positive carrier sets, confirm at least 20% by Gram stain, and a minimum of 4 positive carrier sets by Gram staining, solid media, and VITEK™ analysis (see SOP QC-16, VITEK: Culture Identification Numbers and SOP QC-22, VITEK 2 Compact) to ensure the identity of the organism. Again, if both tubes are positive in a carrier set, only one tube (preferably the secondary subculture tube with carrier) is selected for

confirmatory testing.

10.9.3 Gram stain reactions, cell morphology, and colony characteristics on solid media are given in SOP MB-02, Test Microbes.

10.9.4 Gram stains are performed on smears taken from the positive culture tubes. For the additional confirmatory tests, a loopful of broth from each selected culture tube is streaked on both TSA and selective media appropriate for the test organism and incubated for 18-24 hours at 36 ± 1°C. The selective agar is checked for the correct reaction and the culture on the TSA plate is used for preparing the inoculum for the VITEK™ analysis.

10.9.5 The VITEK™ analysis should be performed according to the manufacturer=s instructions.

10.9.6 If confirmatory testing determines that the identity of the organism was not the test organism, the positive entry (+) on the results sheet must be annotated to indicate a contaminant was present.

10.10 Re-use of Stainless Steel Carriers:

10.10.1 After use, all carriers are autoclaved. Carriers for which test results were negative may be reused after cleaning. Carriers that are positive are recleaned and screened biologically (see SOP MB-03, Screening Carriers) before re-use. These carriers may be reused if the biological screening test results in no growth. The extra carriers that were inoculated but not used are autoclaved, recleaned, and used again.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms (see 16.0). Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP=s official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Strict adherence to the protocol is necessary for the validity of the test results. Any deviation from the standard protocols must be recorded on the form and an explanation for the deviation given.

15.0 REFERENCES:

15.1 Official Methods of Analysis. 2006. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Methods 955.15 and 964.02).

16.0 FORMS AND DATA SHEETS:

16.1 AOAC Use-Dilution Test: Time Recording Sheet for Carrier Inoculation Steps

16.2 AOAC Use-Dilution Test: Time Recording Sheet for Carrier Transfers

16.3 AOAC Use-Dilution Test Information Sheet

16.4 AOAC Use-Dilution Test Results Sheet

16.5 Test Microbe Confirmation Sheet

Attachment A: Testing Footnotes and Explanations

AOAC Use-Dilution Test: Time Recording Sheet for Carrier Inoculation Steps

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Product Reg. No.	
Product Name	
Sample No.	
Organism	

Initials/Date	Test ID	Inoculum Settle Time*		Carrier Seeding Time*		Carrier Dry Time*	
		Start Time	End Time	Start Time	End Time	Start Time	End Time
		/	/	/	/	/	/
		/	/	/	/	/	/
		/	/	/	/	/	/

*Recorded from laboratory clock/and timer.

Comments:

AOAC Use-Dilution Test: Time Recording Sheet for Carrier Transfers

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Product Reg. No.	
Product Name	
Sample No.	
Organism	

Initials/Date	Set	Drop Interval	Carrier Drop Start Time (into the disinfectant)		Carrier Drop End Time (into the primary subculture/neutralizer media)		Carrier Transfer (into secondary subculture)
			Clock	Timer	Clock	Timer	Start Time[1]
Comments:							

1 Carrier transfer into secondary subculture (time elapsed after last carrier dropped in primary); taken from clock

AOAC Use-Dilution Test Information Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments:	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by:_____			
H2O Hardness ($CaCO_3$) ppm	Specified	Titrated (Buret)/Date/Init.	HACH/Date/Init.
[redacted]			
Use Dilution	Specified	As Prepared/Date/Init.	
[redacted]			
Organic Soil	Specified	As Prepared/Date/Init.	
[redacted]			
Neutralizer	Specified		
[redacted]			
Temperature (ºC)	Specified	Chiller Unit Display	Test Tube Water Bath
[redacted]		Before: After:	Before: After:
Contact Time	Specified	As Tested	
[redacted]			
Other Parameters	Specified		
[redacted]			

TEST MICROBE INFORMATION/Confirmed by:_____				
Test Microbe		48-54 Hour Culture		
Org. Control No.			Initiated	Harvested
Avg. CFU/Carrier		Date/Time		

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

AOAC Use-Dilution Test Results Sheet

OPP Microbiology Laboratory

PRODUCT INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.			

CARRIER INFORMATION (to be completed by Analyst)		
Carrier Drop Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date Recorded/Initials									
Primary Subculture / Secondary Subculture (carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary			Number of Carrier Sets with Growth						
			Number of Carrier Sets without Growth						
Viability Controls (Record growth as "+", no growth as "0"): ___ Primary ___ Secondary Acceptable: __Yes __No									
Modifications/Comments:									

Test Microbe Confirmation Sheet
OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.		Comments:	

Source: Tube/Plate ID	Date/ Initials	Stain Results[1]	Media Information			Results		
			Type	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	Vitek ID (if applicable)

[1]Record Gram Stain results as GPC=Gram positive cocci or GNR=Gram negative rods.

Attachment A:

Testing Footnotes and Explanations
OPP/Microbiology Laboratory

Footnote	Description
A	Indicates that the seeded carrier, hook, or forceps hit the interior sides of the medication tube containing disinfectant as the carrier was being dropped.
B	Indicates that the carrier was lost (dropped) during a transfer and was not recovered.
C	Indicates that a tube of a positive carrier set (one showing growth) was later determined to be a contaminant and not the test microbe. In AComments@ refer to the confirmation information for details.
D	Indicates that the primary or secondary subculture tube containing the carrier broke during vortexing. In the AComments@ indicate if carrier was recovered or if the remaining broth was placed in another tube.
E	Indicates that the carrier was exposed to the disinfectant late or early, outside of the +/- 5 second drop, spray, or wipe interval. In AComments@ indicate the approximate number of seconds outside (+/-) of the 5 second interval.
F	Indicates that the carrier was placed in the neutralizer late or early, outside of the +/- 5 second drop interval. In AComments@ indicate the approximate number of seconds outside (+/-) of the 5 second interval.